サイクリン依存性キナーゼ5/P25阻害活性を有するチエニル・トリアゾール誘導体のコンフォメーション解析

Conformational Analysis for a Series of Thienyl Triazoles Derivatives that Act as Cyclin-Dependent Kinase 5/P25 Inhibitors

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Abstract

A conformational analysis study has been performed for a series of 48 triazolyl-thienyl derivatives active against cyclin-dependent kinase 5/p25 (cdk5/p25) with the purpose of identifying the local and global minima on the potential energy surface and possibly, to predict the bioactive conformation of the most active compounds of the title series. The exhaustive sampling has been carried out in gas phase and aqueous environment using the serial torsional sampling method implemented in Macromodel module from Schrödinger suite. The obtained conformers have been minimized by the OPLS_2005 force field using the Polak-Ribiere conjugate gradient method with RMS gradient equal or less than 0.01 kcal/Å·mol as stop criterion in the optimization process. The number of rotatable bonds which have been varied during the conformational search ranges between 4 and 7. The conformational preferences of this series of compounds are mainly ruled by the stabilizing effect of π-electron delocalization, a planar geometry being favored. Also, the comparison of potential energy values obtained from gas and water based conformers has shown the values corresponding to water conformations are smaller than their gas counterparts.

Keywords: conformational analysis, Macromodel, triazolyl-thienyl derivatives, cdk5/p25 inhibitors, Schrödinger suite

1. Introduction

Serine/threonine kinases are a large family of proteins involved in numerous physiological processes, e.g. cell cycle regulation, transcription, neural functions and apoptosis, and their deregulation is associated with a plethora of disorders. Among them, Alzheimer disease's (AD) is one of the most widely spread worldwide, with more than 5 million people living with

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it in US alone (Alzheimer's association, http://www.alz.org/alzheimers_disease_what_is_alzheimers.asp). Although, a great deal of effort has been invested in the development of a drug to address this brain disorder, an effective cure has not yet been developed.

Several studies have reported a serine/threonine kinase, namely cyclin-dependent kinase 5 and its cofactor p25 (cdk5/p25), to be one of the major players in the development of AD (Lau et al., 2002) and other neurodegenerative disorders such as Parkinson's disease, Huntington's disease and stroke (Smith et al., 2003; Dhavan et al., 2001). The accumulation of p25 (Patrick et al., 1999) and increased cdk5 activity (Lee et al., 1999) has been observed in the brains of AD's patients. These findings have led to an active search for compounds to inhibit the abnormal cdk5/p25 complex for treating of AD. In this endeavor, numerous classes of potent chemical compounds have been discovered (Leclerc et al., 2001; Glicksman et al., 2007; Sridhar et al., 2006), such as 2-aminoethyl derivatives (Dhavan et al., 2001; Kim et al., 2002; Misra et al., 2004a; Misra et al., 2004b), paullones (Leost et al., 2000) and 4-aminoimidazole derivatives (Helal et al., 2009), as potential inhibitors of cdk5/p25 for the treatment of AD and other neurodegenerative diseases. Few examples of well-known cdk5 inhibitors are displayed in Figure 1.

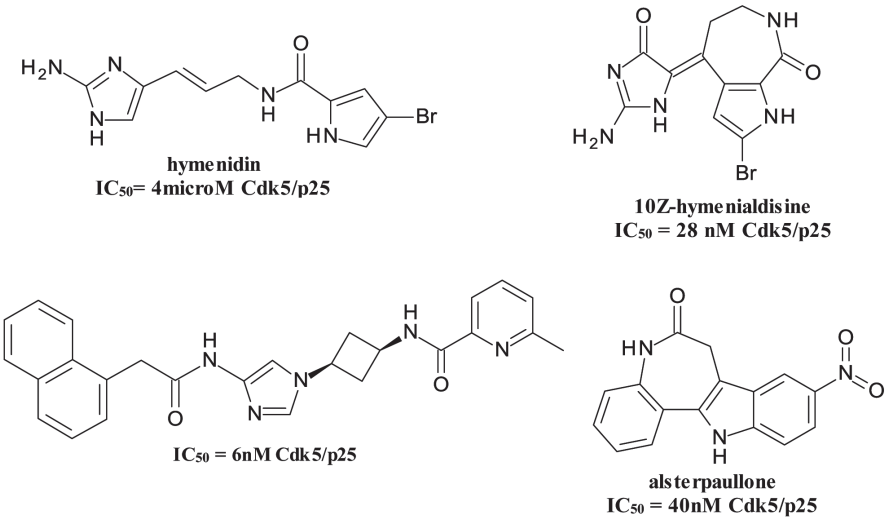


Fig. 1 Representative inhibitors of cdk5/p25

Recently, thienyl triazoles derivatives have been reported as potent and selective inhibitors for cdk5/p25. Combined studies of SAR and directed synthesis have led to the identification of a novel series of clubbed triazolyl-thienyl derivatives (Shiradkar et al., 2007) as potent cdk5/p25 inhibitors. This potential activity and selectivity may turn these compounds into promising lead structures which can be used as tools for the study and possibly treatment of AD, particularly and neurodegenerative and proliferative disorders, in general.

Due to their biological potential, we were interested to explore the conformational space of

these compounds and identify the specific conformations which might be responsible for the biological behavior of this class of compounds. Relevant information about the bioactive conformation of a compound can be obtained from conformational analysis, a valuable tool which offers information regarding the geometrical features of the active and inactive compounds and the conformations corresponding to possible global minima of the conformational energy surface. Employing this method, the total number of possible conformers in low minima populating the potential energy surface (PES) can be evaluated and the energy necessary for different conformational changes can be estimated.

In order to find a set of low-energy conformers and to establish the main characteristics for the lowest-energy conformer ensembles for the series of ligands taken for study, a conformational analysis in both vacuum and aqueous environment was performed. The results of this study are needed for further molecular modeling studies employing the title series of compounds, e.g. pharmacophore searches, 3D-QSAR, molecular docking, etc.

2. Methods

The 3D coordinates of the compounds were generated from the SMILES codes. The resulted structures were energy minimized using the OPLS_2005 force field with Polak-Ribiere conjugate gradient method implemented in Macromodel 9.6 (Schrödinger, LLC, New York, NY, 2008). To reach the convergence, a RMS gradient equal or less than 0.01 kcal/Å·mol was set up. The minimized structures were further used as input for the conformational search performed in vacuum and continuum solvent (water) with Monte Carlo Multiple Minimum method implemented in Macromodel 9.6. This method, also known as the torsional sampling method, is highly efficient in performing global searching, exploring close as well as distant areas of the potential energy surface (PES). By default the constant dielectric was set to 1 and the search was carried out until 1000 conformations were found. The resulted conformers were minimized for up to 500 steps using the conjugate gradient method with the above mentioned parameters.

Besides, the conformational analysis was carried out in water, because nearly all experimental studies are achieved in solvent. The calculations were performed using the same conditions as for vacuum conformational search.

3. Results and Discussions

The title series of triazolyl-thienyl derivatives comprises 48 ligands acting as selective and effective inhibitors of cdk5/p25 (Shiradkar et al., 2007). All ligands share a substituted thienyl ring and a rigid core of variable size. The number and size of the fused ring system was used as principal element in dividing the set in four groups. The general structures are presented in Figure 2.

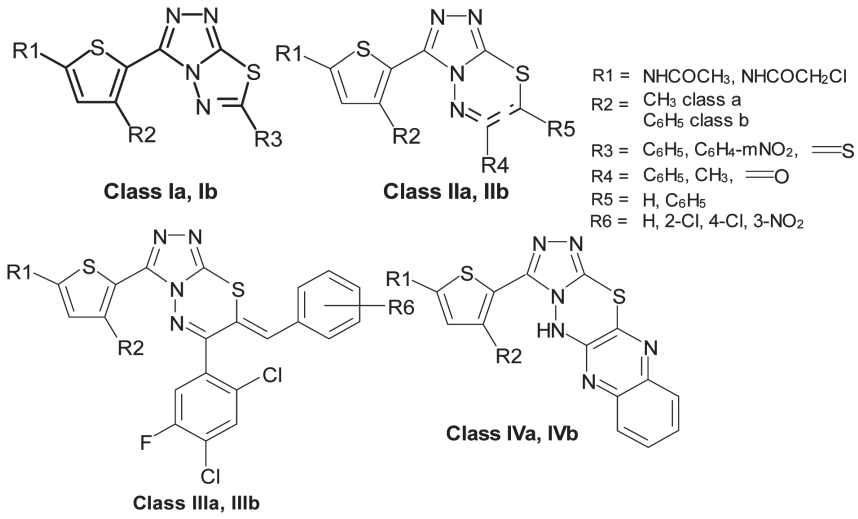


Fig. 2. Generic structures of triazoles derivatives

The conformational analysis has been carried out for all these ligands having two main purposes in mind: firstly, to thoroughly explore the conformational space occupied by the entire series of ligands and identify the geometric features of global minima, possible bioactive conformation and secondly, to investigate the influence of environment in computing the lowest and highest energy conformations.

To accomplish these tasks the conformational search was performed in vacuum and aqueous environment. The importance of considering solvent effects in conformational energy calculation was pointed out in previous studies (Boström et al., 1998; Perola and Charifson, 2004). This observation prompted us to analyze and compare the results obtained from both environments with the aim of assessing possible differences between them.

A slightly difference has been noticed in the number of conformers generated per compound. Overall, 54% of the title series, namely 26 ligands, give a greater number of conformers in solvent when compared with vacuum (Figure 3A). However, a larger number of conformers per compound were generated in vacuum as can be seen from the median value of the number of conformations calculated per ligand (Tabel 1). On the other hand, the median values for relative energies (Erel - computed as difference between the energy of the lowest and highest energy conformers for each ligand) and computed energy are favorable to the calculations performed in solvent.

Table 1. Median values of relative energies

State	No confs*	E_{rel} (kJ/mol)	E_{OPLS} (kJ/mol)
Vacuum	23	15.25	101.45
Solvent	19	18.07	22.72

* No confs – median values for the number of conformers calculated per ligand

The relative energies calculated for each ligand with respect to the global minima ranged from 4.22 to 20.994 kJ/mol when calculations were performed in solvent (Erel_w) and from 1.011 to 20.995 kJ/mol when the calculations were performed in vacuum (Erel_v) (Figure 3B). The median relative energies were 18.068 and 15.251 kJ/mol, respectively (Tabel 1). This explains the greater number of conformers generated in vacuum when compared with the conformers generated in solvent (Figure 3A).

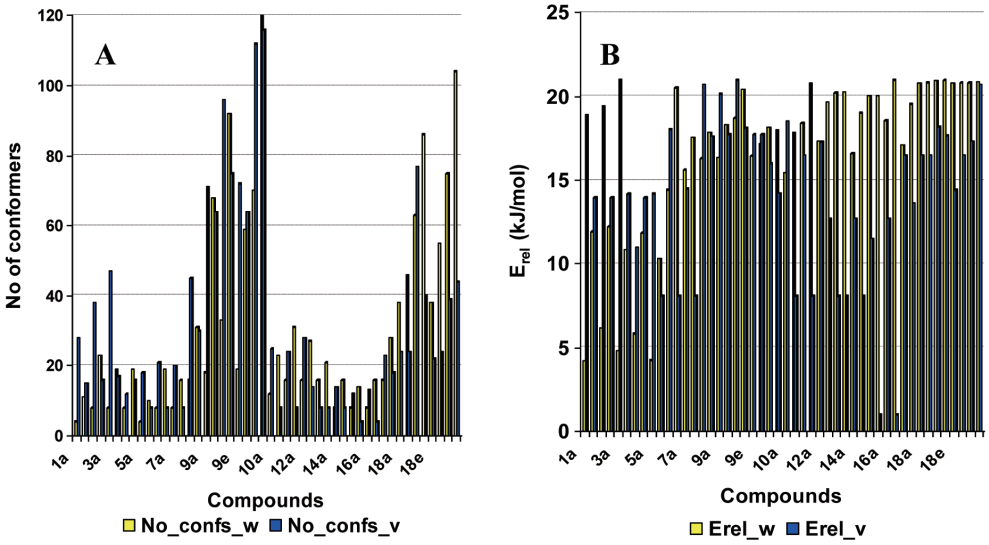


Fig. 3 Distribution of the number of conformers (panel A) and relative energies (panel B) calculated in solvent (w) versus vacuum (v)

Conversely, the potential energies of the lowest energy solvent conformers are smaller than the energy values corresponding to the same conformers generated in vacuum. This trend was noticed, without exception, for all compounds of the title series (Figure 4).

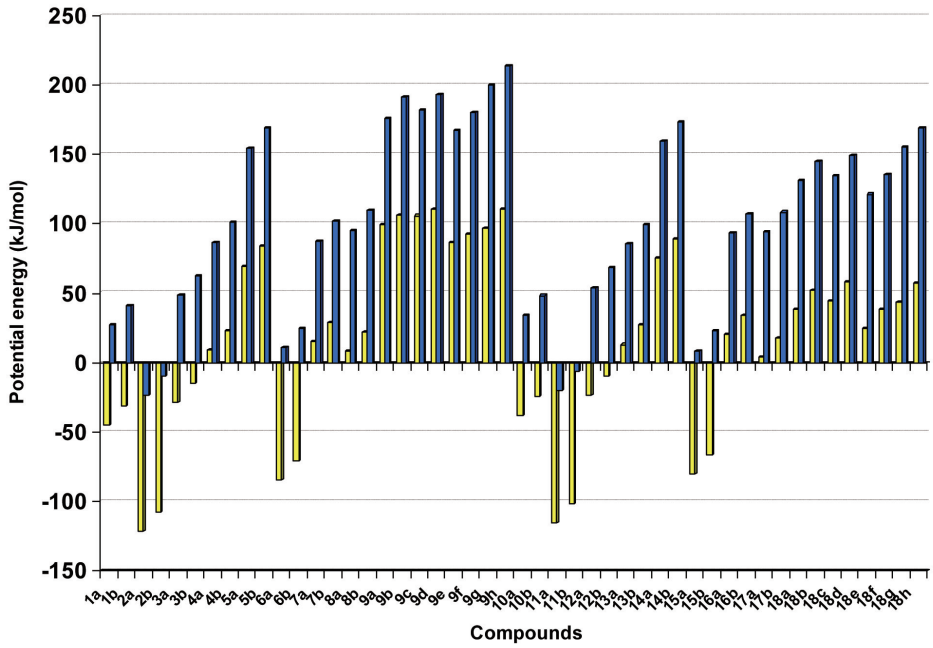


Fig. 4 Distribution of potential energies for the conformers calculated in solvent (E_w, depicted as yellow bars) and vacuum (E_v, depicted as blue bars)

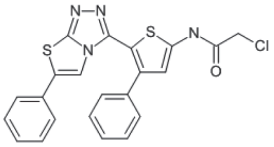
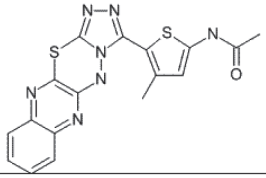
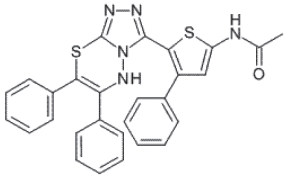
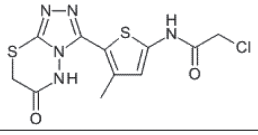
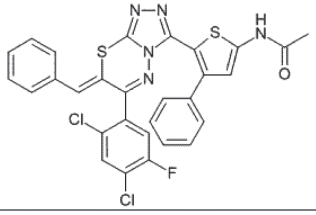
A comparison between the potential energies of the lowest energy conformations calculated in vacuum and solvent for the most potent ligand of each class is presented in Table 2.

A possible explanation (Perola and Charifson, 2004) for the observed differences would be that when calculations are performed in vacuum internal electrostatic interactions are magnified, relative to the solution state. This leads to folded conformations characterized by intramolecular interactions between topologically distant atoms involving at least one charged group. Consequently, collapsed conformations of higher energy are generated. On the other hand, in solution state the polar or charged groups are solvated and stabilized in H-bonds with the solvent molecules and it is necessary to desolvate them in order to interact to each other.

Concerning the geometries of the global minima calculated for both environments, these share some common features, but display different characteristics, too. The geometries of the lowest and highest energy ensembles for all 4 groups of analyzed ligands are depicted in Figures 5 and 6.

Regardless of the environment, the substituted thienyl ring, common to all ligands of the title series (Figure 2), defines the same plane with $NHCOCH_2X$ moiety, while the variable size fused-rings system adopts an out of plane orientation. Consequently, the dihedral θ_1 has

Table 2. Energies calculated in vacuum and solvent for the global minima of the same ligand

Compound	Class	IC_{50} (nM)	E_{OPLS_V} (kJ/mol)	E_{OPLS_W} (kJ/mol)
	Ib	28	107.35	33.8
	IVa	34	154.75	69.73
	IIb	40	85.39	12.98
	IIa	820	-9.86	-107.89
	IVb	1452	131.31	38.33

a value around ± 40° (± 4°) for the global minima corresponding to all classes of ligands (Figure 5 and 6 – panels A,B,E, and F), while a larger set of values were encountered for the highest energy conformers, ranging from ± 30° to ± 150° (Figure 5 and 6 – panels C, D, G, and H).

Significant differences between the geometries of conformations generated in solvent and vacuum were noticed for the orientation of $NHCOCH_2X$ moiety (where X=H, Cl). Thus, two distinct planar orientations of $NHCOCH_2X$ susbtituent with respect to the thienyl ring were noticed, when the geometries of global minima calculated in solvent (Figure 5 – panels A and E) and vacuum (Figure 5 – panels B and F) were compared. The first orientation corresponds to a value of dihedral θ_2 of 0° and it is characteristic for global minima calculated in solvent (Figures 5 and 6 – panels A and E), while the value of θ_2 around -180° corresponds to vacuum global minima (Figure 6 – panels B and F). Furthermore, dihedral θ_3 can have

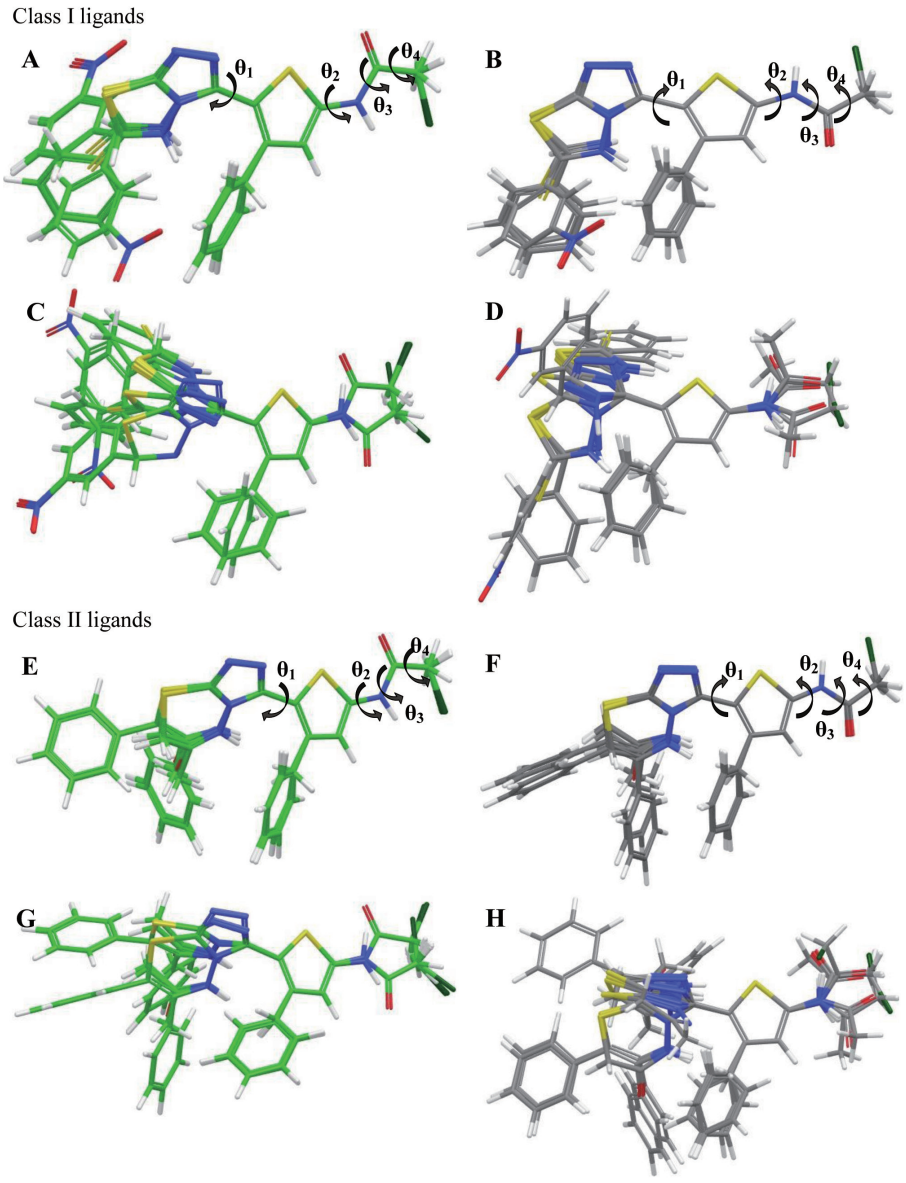


Fig. 5 The geometries of the lowest and highest energy conformations calculated in solvent (figure' s left side) and vacuum (right side) exemplified for class I (panels A, B, C, and D), and class II ligands (panels E, F, G, and H). For simplicity, the dihedrals varied during the conformational analysis are depicted only for the lowest energy conformers.

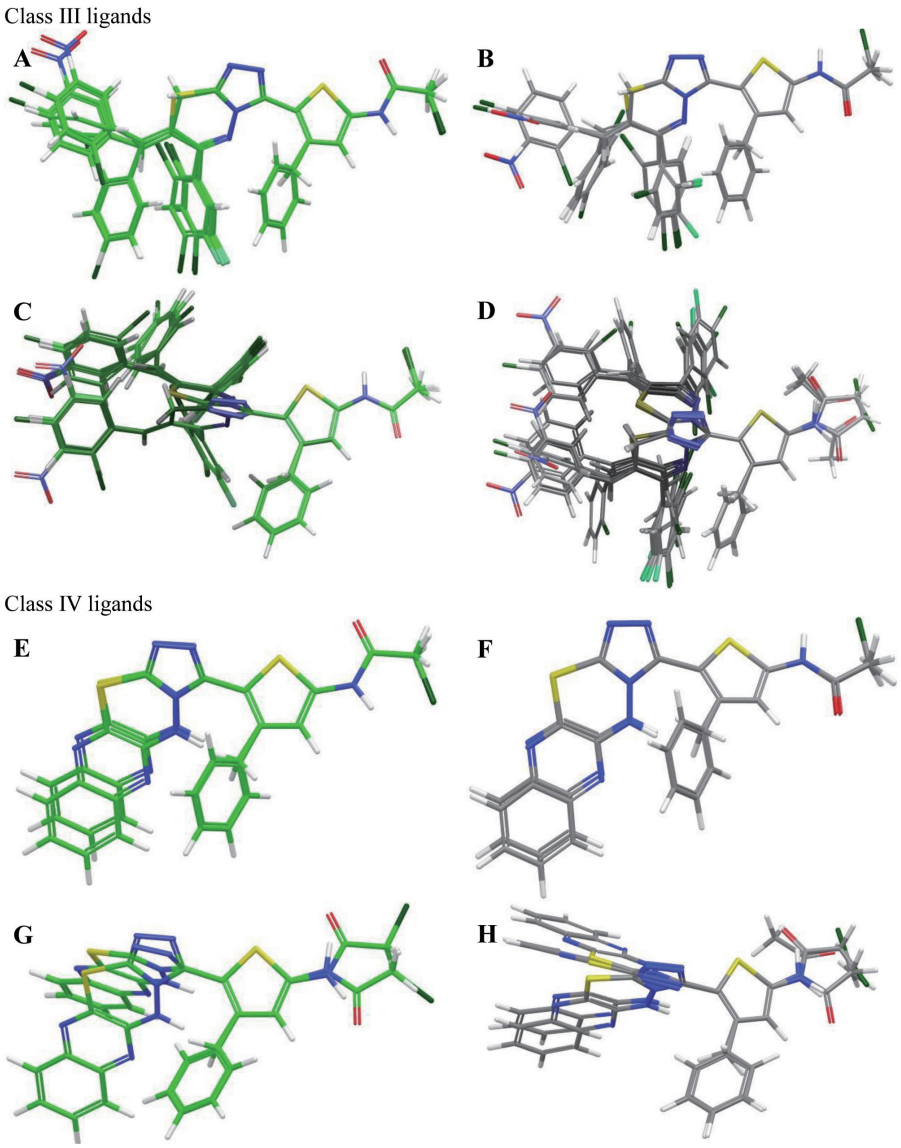


Fig. 6 The geometries for the lowest and highest energy conformations calculated in solvent (figure' s left side) and vacuum (right side) exemplified for class III (panels A, B, C, and D), and class IV ligands (panels E, F, G, and H).

only two values ± 180º (± 1º), while θ_4 is around 0º (± 3º), regardless of the environment. These values are characteristic for the lowest energy conformations corresponding to all classes of ligands.

In the case of higher energy conformations different orientations of NHCOCH2X moiety were observed and accordingly, a larger range of values for dihedrals θ_2 and θ_3 was noticed, especially for conformations calculated in vacuum (Figures 5 and 6 – panels D and H).

4. Conclusions

The results of this study provide insights on the conformational behavior of this class of compounds and offer useful information about the geometries in different environments.

The relative energies of conformations calculated in solvent are slightly higher than the corresponding values of the conformations calculated in vacuum, while the OPLS energy are considerable lower in solvent when compared with the conformations of the same ligand calculated in vacuum. This pattern has been noticed for the entire series and it could be explained by the amplification of internal electrostatic interactions in vacuum relative to the aqueous environment. A "folded" conformation is favored in vacuum having the distance between the sulphur of thienyl and amidic H of 2.5 Å, while the distance between amidic O and the same sulphur atom is larger than 3 Å for the lowest energy conformations calculated in solvent.

As it was expected this difference correlates with the geometries calculated in both environments, which differ significantly.

In all compounds, the preferred arrangement of the central core comprised of the thienyl ring and $NHCOCH_2X$ (X=H, Cl) moiety, is planar with two different orientations for the global minimum in solvent and vacuum.

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要　旨

サイクリン依存性キナーゼ 5/P25 阻害活性を有する チエニル・トリアゾール誘導体のコンフォメーション解析

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サイクリン依存性キナーゼ 5/P25 に対して阻害活性を持つ 48 種類のトリアゾリルーチエニル誘導体について、最も活性の高い化合物を設計するためにポテンシャルエネルギー表面の極小点と最小点を探索してコンフォメーション解析を実施した。高性能な分子設計シミュレーション・ツールである「Schrödinger suite」にあるマクロモデルモジュールを用いて、各分子の気相および水中での安定なコンフォメーションを検討した。それにより得られたコンフォメーションのエネルギーを最小化するように、Polak-Ribiere 勾配法を用いた OPLS_2005 力場によって、RMS 勾配度が 0.01 kcal/Å·mol 以下で収束する条件で探索した。コンフォメーションの探索においては、回転可能な結合数を 4 から 7 の間で変化させた。エネルギー的に好ましいコンフォメーションは、主として非局在 π 電子の安定化であり、平面構造が重要であることが分かった。また、気相および水中でのポテンシャルエネルギー値の比較から、水中でのコンフォメーションの方が気相中でのコンフォメーションよりも安定であることが明らかになった。